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Neural Control of the Lateral Abdominal Arterial Valves in the Lobster *Homarus americanus*

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Abstract. A dorsal abdominal artery in *Homarus americanus* runs the length of the abdomen, giving rise to one pair of large lateral arteries in each segment. These lateral arteries supply hemolymph to the abdominal muscles and the swimmerets. In addition, many small vessels leave the dorsal abdominal artery ventrolaterally to supply the gut and gonads. Bicuspid muscular valves are located at the junction of each segmental lateral artery with the dorsal abdominal artery, but not at the origin of the gut vessels. Nerves originating from the ventral abdominal ganglia travel along the lateral arteries to innervate the valves, providing both inhibitory and excitatory inputs. Inhibitory input produces hyperpolarizing inhibitory junctional potentials that relax the valve muscles, and in intact *in situ* perfused arteries causes increases in outflow from the affected lateral artery. Excitatory input produces depolarizing excitatory junctional potentials that close the valves and reduce perfusate outflow. The valve nerves also branch to innervate valves up to two segments anterior and one segment posterior. Application of exogenous γ-aminobutyric acid hyperpolarizes valve muscle fibers. This and the hyperpolarizing effect of valve nerve stimulation are reversibly abolished by the application of picrotoxin (10^{-5} *M*). Acetylcholine (10^{-5} *M*), but not glutamate, causes depolarization and contraction of valves. The role of the valves in controlling the distribution of hemolymph flow is discussed.

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Abbreviations: Ach, acetylcholine; DAA, dorsal abdominal artery; EJP, excitatory junction potential; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; IJP, inhibitory junction potential; LAA, segmental lateral abdominal artery; LAAV, lateral abdominal arterial valve.

Introduction

In decapod crustaceans, seven arteries deliver hemolymph from the heart to the systemic circulation. These arteries branch into progressively finer vessels in the tissues until they open into small capillary-sized vessels that are bounded by a thin intima (Martin and Hose, 1995). The smallest vessels are the functional equivalent of vertebrate capillaries, and it is here that exchange of metabolites, gases and ions occurs between the hemolymph and the tissues.

The crustacean circulation is an open system, lacking a tubular venous return to the heart. Instead, "venous" hemolymph collects into progressively larger sinuses and is returned to the heart *via* the gills. Despite very early observations on the anatomy and innervation of various flow-rectifying structures, such as cardioarterial (CA) and segmental lateral arterial valves of the dorsal abdominal artery (Alexandrowicz, 1932a), the control of hemolymph circulation in crustaceans has been poorly understood until recently. Inhibitory innervation of the sternal CA valve in *Homarus americanus* causes valve relaxation and increased outflow in this artery (Kuramoto *et al.*, 1992, 1995). The 11 CA valves in the isopod *Bathynomus doederleini* receive varying patterns of innervation (Kihara *et al.*, 1985), and the activity of the inhibitory nerve to the CA valve of the artery supplying hemolymph to the swimmerets is coordinated with the activity of the swimmerets themselves (Fujiwara-Tsukamoto *et al.*, 1992).

Changes in regional blood flow have been measured in intact *Cancer magister* using ultrasonic techniques (Airriess and McMahon, 1994, 1996; McGaw *et al.*, 1994). During hypoxia, hemolymph is diverted away from the viscera and brain and toward the locomotory and ventilatory appendages. Injection of dopamine into intact nor-

moxic *C. magister* produces a similar response, suggesting hormonal control of hemolymph distribution. However, the sites of regulation of hemolymph flow distribution were not identified in these animals. In *H. americanus,* a number of neurotransmitters and hormones, with peptides being the most effective, have been shown to alter the peripheral resistances of *in situ* perfused arterial trees (Wilkens, 1997). Two possible mechanisms for regulation of regional blood flow in decapods exist: (1) the centrally located muscular CA valves may differentially regulate blood flow in arteries leaving the heart, as seen in *B. doederleini* and *Panulirus japonicus* (Kuramoto and Ebara, 1984, 1989), and (2) the relative resistances of arterial pathways may be altered by peripheral mechanisms.

How can the peripheral resistance of arteries be controlled? Unlike the blood vessels of vertebrates, the walls of all crustacean arteries, except the dorsal abdominal artery (DAA), lack muscle layers. A layer of striated muscle fibers has been found in the DAA of a number of macruran species including *H. americanus* (Burnett, 1984; Martin *et al.,* 1989; Wilkens *et al.,* 1997a). Since hormones and nervous inputs cannot act directly on vessels that lack muscular walls, other mechanisms for controlling arterial resistance and regional hemolymph flow must exist. Muscular arterial valves have been identified at the junction of each lateral abdominal artery (LAA) with the DAA in *Panulirus interruptus* (Alexandrowicz, 1932a). In decapod crustaceans, the DAA supplies blood to the abdominal positioning muscles and swimmeret muscles via the LAAs, and to the gut through many small arterioles that arise from the ventral wall of the DAA. The lateral abdominal arterial valves (LAAV) may be the sites where the distribution of hemolymph is differentially regulated between the locomotory and vegetative organs. This paper investigates the role of the LAAVs in controlling hemolymph flow in this part of the circulation in *H. americanus.*

Materials and Methods

Lobsters (approximately 500 g) were purchased from a commercial supplier and maintained in a recirculating seawater system at 12°C prior to experimentation. Animals were fed frozen smelt twice weekly. Data presented here are based on observations taken from 12 animals.

In preparation for surgery, lobsters were restrained with elastic bands and cold-anesthetized by burial in crushed ice in an acrylic plastic box (50 × 20 × 20 cm) for 20 to 30 min. While a lobster was still buried in ice, its hemolymph was replaced by saline (Cole, 1941, pH = 7.6). To exsanguinate an animal, the heart was exposed by removing the overlying dorsal carapace and dermis. Ice-chilled saline was pumped through a polyethylene cannula, tipped with a 21-gauge needle, directly into the ventricle *via* an ostium. The heart continued to pump slowly, and hemolymph returning to the pericardium was allowed to escape into the container. Perfusion was continued until all traces of hemolymph were removed. Following exsanguination, the ice was replaced with artificial seawater at 12°C. The dorsal cuticle was removed from each abdominal segment and the DAA and proximal sections of each LAA were exposed by dissecting away the dermis and superficial abdominal positioning muscles.

In situ *preparations*

The DAA was cannulated *in situ* at a point close to the bulbus arteriosis with polyethylene tubing (PE 160), and the cannula was secured with surgical silk. A peristaltic pump was used to perfuse the artery with saline at a flow rate of 1.5–2.5 ml min^{-1}. To measure pressure in the DAA, a small transverse incision was made in the dorsal wall of the artery, posterior to the branching point of the third pair of LAAs. A saline-filled polyethylene cannula (PE 160), previously heated and drawn to about one-third of the arterial diameter, was inserted through the slit and secured with surgical silk. This cannula was connected to a Hewlett-Packard pressure transducer (267BC) and amplifier (311A). All of the LAAs except one were tied off with surgical silk. A small incision was made in the dorsal wall of the LAA remaining patent. A short length of polyethylene cannula drawn to a taper and connected to an ultrasonic flow transducer (Transonics, 1 mm i.d.) was inserted into the LAA through the incision and secured with surgical silk. The flow probe was then connected to a Transonics (T106) flow meter.

The effects of stimulating the nerves to the valve of the patent LAA and of various test compounds on LAA flow and DAA luminal pressure were measured. The LAAV nerves were stimulated as described below. To apply drugs, the perfusate (2.0 ml min^{-1}) was switched from saline to one containing the desired compound. After application of a drug, the preparation was perfused with regular saline until all variables had recovered to pretreatment levels. The drugs tested were acetylcholine (ACh), γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT) and picrotoxin (Sigma).

In vitro *preparations*

Following exsanguination, the DAA was separated from the underlying gut and gonads by cutting the fine arterioles that arise from its ventrolateral surface to supply these organs. Each lateral artery was cut 0.5–1.0 cm from its junction with the DAA. The DAA with attached proximal sections of the LAAs was placed in an acrylic tissue bath (volume 15 ml) lined with Sylgard and filled with aerated saline at 12°–15°C. The vessel was cannulated

anteriorly using a polyethylene cannula as described above and flushed by pumping saline at a flow rate of 4–5 ml min^{-1}. Next, the artery was secured by micropins placed in the severed ends of the LAAs, the cannula was removed, and the DAA was opened longitudinally by cutting along the dorsal midline. The DAA was pinned open with the luminal surface uppermost, exposing the valves (see Fig. 1).

The LAAV nerves were stimulated using a glass suction electrode (tip diameter 50 μm) and a Grass S48 stimulator. Trains of impulses (1-ms duration) were applied to the nerve at varying frequencies (1–80 Hz). Fluctuations produced in the membrane potential of individual valve muscle fibers by nerve stimulation were recorded intracellularly. Glass microelectrodes (tip resistance 15–25 MΩ) were pulled from thin-walled borosilicate glass tubing. These were filled with 3 *M* KCl and connected to a WPI M4-A electrometer. Signals from the electrometer were displayed on a Tektronix 5103N oscilloscope and recorded on a Vetter 420L four-channel video recording system. Hard copy was printed with a Gould 2400 chart recorder. Between recordings, the vessel was superfused at a flow rate of 2.5 ml min^{-1} with chilled saline to maintain the bath temperature between 12° and 15°C.

The effects of various drugs on the membrane potential of valve muscle were tested by direct application to the bath over an exposed valve. Prior to testing, all solutions (drug in saline) were maintained at the same temperature as the bath. In random order, 0.5 ml of each solution was applied with a pasteur pipette. The bath was perfused with fresh, aerated saline after each drug.

Morphology

The orientation of LAAV muscle bundles and the innervation of the valves were examined using a Wild M-5 stereomicroscope. Nerve tissue was stained for 24–48 h at 4°C with methylene blue (10 drops of 0.1% stock added to 100 ml of saline). Fading of the stain upon exposure to light was prevented by placing tissue samples in 4% ammonium molybdate in saline at 4°C for 1 h prior to examination.

Results

Morphology

The DAA originates from the posterior wall of the bulbus arteriosus of the heart and passes along the dorsal midline of the abdomen. The diameter of the vessel decreases posteriorly. Within each of the first five anterior

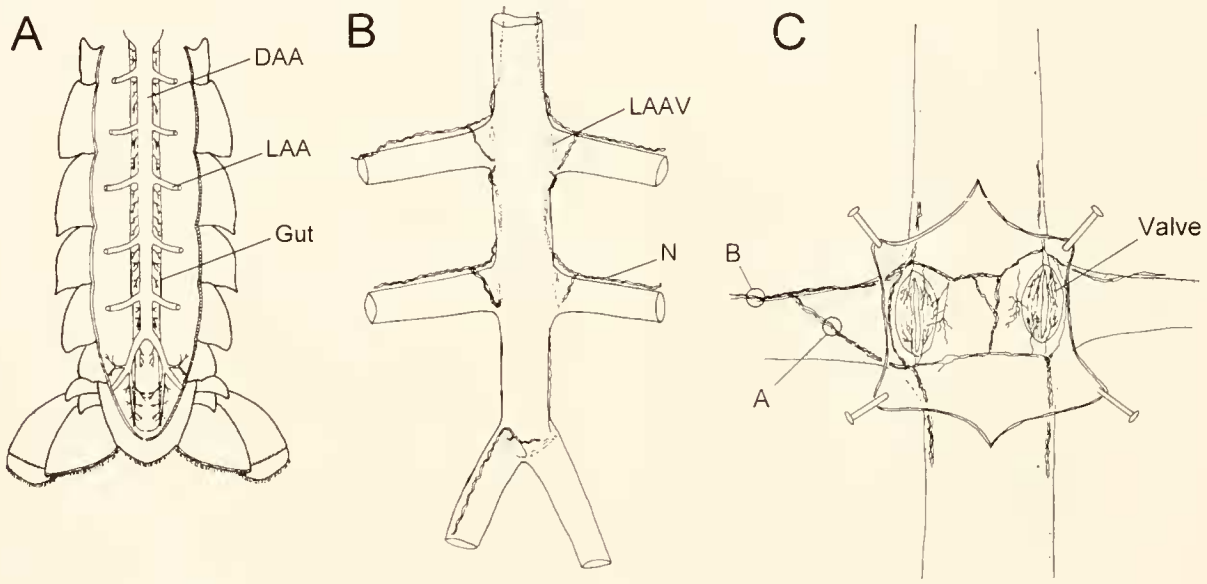

Figure 1. (A) Dorsal view of the dorsal abdominal vasculature in *Homarus americanus*. The dorsal abdominal aorta (DAA) lies along the dorsal surface of the hindgut (Gut). Within each of the first five abdominal segments, a pair of lateral abdominal arteries (LAA) branch off the DAA at roughly right angles. The bifurcating pair of arteries at the posterior margin of the fifth segment are homologous to the more anterior LAAs. (B) The innervation patterns of the LAA valves. The valve nerves (N) approach each valve in connective tissue along the anterior edge of each LAA. (C) A dorsal view of the junction of one pair of segmental lateral abdominal arteries with the dorsal abdominal aorta. The dorsal wall of the DAA has been cut longitudinally and the flaps have been pinned back to reveal the lateral abdominal artery valves (Valve). A and B indicate the sites of nerve stimulation proximal and distal to the point where the incoming nerve bifurcates.

abdominal somites, a pair of LAAs branch at roughly right angles from the DAA (Fig. 1A). Each LAA extends laterally for a short distance, then descends and arborizes to supply hemolymph to the abdominal muscles and the swimmerets (Wilkens *et al.*, 1997b). Branches of the LAAs also supply the gonads. At the posterior margin of the 5th abdominal segment the DAA bifurcates at an acute angle into two smaller arteries that supply the uropods and telson. These two arteries are the modified LAAs of the 6th abdominal segment. A bicuspid muscular valve is situated at the junction of each LAA with the DAA, and also at the junctions of the two posterior arteries with the DAA. The valve muscle fibers lie in an anteroposterior direction, and the valve aperture appears as a horizontal slit. A rich array of very small vessels arises from the ventrolateral wall of the DAA to supply hemolymph to the gut and posterior portions of the gonads. No valves exist at the origins of these gut vessels (Wilkens, 1997).

Segmental nerves, embedded in connective tissue, approach each LAAV along the anterior edge of each LAA. The valve nerves originate from the second root of each abdominal ganglion in the next anterior segment. Methylene blue staining revealed multiple axons in these nerves (Fig. 1B). As the nerve approaches a valve, it bifurcates. One branch continues to follow the anterior edge of the LAA until it reaches a dense cluster of axons at the anterior edge of the valve. The second branch passes posteriorly over the dorsal surface of the LAA to a cluster of axons at the posterior edge of the valve. The distance of the point of bifurcation from the valve was quite variable. In some instances the bifurcation was several millimeters away from the valve, whereas in others it occurred at the junction of the LAA and DAA. Proximal to the bifurcation, both branches appeared to contain three axons (Fig. 1C). Two of the three axons arise from the distal LAAV nerve proper. The third axon, not originating in the LAAV nerve, spans the bifurcation of the other two axons, passing over the dorsal surface of the LAA. The origin and target of this third axon could not be discerned, but it appeared to link the clusters of axons at the anterior and posterior edges of a particular LAAV. The nerves terminate at synapses on the two muscular flaps of each valve anteriorly, posteriorly, and medially. Contralateral valves within each segment were connected by many small axons traversing the ventral luminal wall of the DAA in a "figure eight" fashion (Fig. 1C). The pattern of innervation described for each abdominal segment was bilaterally symmetrical. Ipsilateral valves in adjacent segments were connected by two to four axons running longitudinally along the external ventrolateral wall of the DAA (Fig. 1B). In the first abdominal segment, the LAA nerves supply the LAAVs of this segment and also pass forward along the DAA to terminate on the sternal cardioarterial valve (SCA).

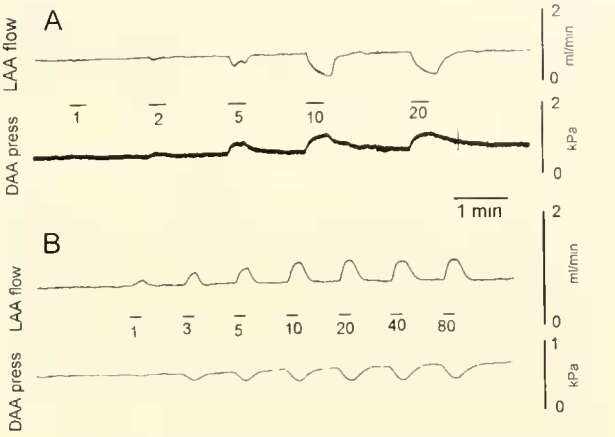

Figure 2. The effect of proximal excitatory (A) and distal inhibitory (B) valve nerve stimulation on LAA flow and DAA pressure in an in situ saline-perfused preparation of the dorsal abdominal vasculature in *Homarus americanus*. The stimulating locations are as shown in Figure 1C. Stimulating frequencies are indicated along each set of records.

The innervation of the two valves located at the posterior bifurcation of the DAA was difficult to visualize, but in one preparation both valves were innervated by a single nerve approaching along the dorsolateral surface of one of the posterior vessels (Fig. 1B), a position homologous to the route of the nerve in the more anterior LAAs.

In situ *preparations*

The valve flaps were clearly visible through the transparent wall of the LAA. Spontaneous rhythmic contractions of LAAVs were frequently observed in saline-perfused arteries. With each contraction, the LAA flow decreased and the luminal pressure in the DAA increased (not illustrated). Conversely, valve relaxation was accompanied by increased LAA flow and a drop in DAA luminal pressure. These pressure fluctuations caused substantial changes in the diameter of the DAA.

Stimulation of the LAAV nerve at a site distal to the bifurcation (site B in Fig. 1C) always resulted in a relaxation of the valve, an increase in flow through that LAA, and a concomitant decrease in DAA lumen pressure (Fig. 2A, typical of 20 observations on six arteries). Stimulation proximal to the bifurcation of the nerve (site A in Fig. 1C) resulted in contraction of the valve, a decrease in LAA flow, and an increase in DAA lumen pressure (Fig. 2B, three arteries). The biphasic change in membrane potential observed during stimulation at A (see Fig. 4C) was not reflected in the LAA flow and DAA pressure responses to stimulation at this site. The maximal change in LAA flow to proximal stimulation occurred at the stimulation frequency of 20 Hz.

Figure 3 demonstrates the effects of the neurotransmitter ACh on flow through the LAA *in situ* ($n = 2$). ACh caused valve closure and a decrease in flow through the LAA.

In vitro *preparations*

The mean resting membrane potential of LAAV muscle fibers was -40 ± 1.7 mV ($n = 12$ fibers from five animals). The effect of LAAV nerve stimulation on the membrane potential of valve muscle fibers is shown in Figure 4. Lateral abdominal arterial valve nerves were stimulated at the two sites marked in Figure 1C: one proximal (site A) and the other distal (site B) to the nerve bifurcation. Stimulation at A did not produce unitary excitatory junction potentials (EJPs), but did produce frequency-dependent summing depolarizations. The rates of onset and recovery of excitatory responses, and their amplitudes, were lower than for inhibitory input. EJPs produced only graded depolarizations; all-or-none action potentials were never observed. EJPs were recorded only in very fresh preparations, with the excitatory response being lost after a short time *in vitro*.

Without exception, stimulation at point B distal to the bifurcation of the LAAV nerve produced inhibitory junction potentials (IJPs) in the muscle fibers of the ipsilateral valve (Fig. 4B, $n = 8$), the contralateral valve in the same segment ($n = 3$), and both the ipsilateral and contralateral valves in the next anterior segment ($n = 6$). IJPs produced at these different sites were usually of similar magnitude. In two preparations, IJPs were also recorded from the contralateral valve two segments anterior. The magnitude of the IJPs in this valve was smaller than in the other four sites (not shown). In one other preparation, IJPs were recorded in the ipsilateral valve one segment posterior. Stimulation of the LAAV nerve at point B in the first abdominal segment produced IJPs in the sternal cardioarterial valve in addition to the ipsilateral and contralateral LAAVs in that segment (not illustrated). In all cases the magnitude of the hyperpolarization at a given site increased with increasing stimulation frequency up to 40–80 Hz, indicating summation of IJPs.

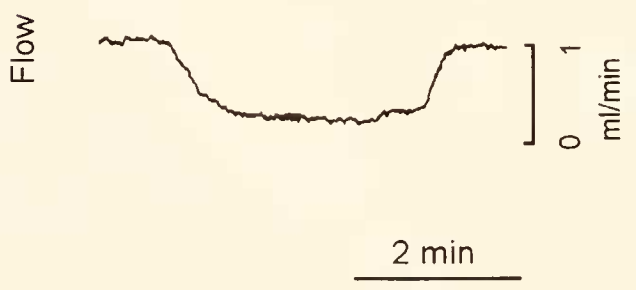

Figure 3. The effect of ACh (10^{-4} *M*) on the flow through a lateral abdominal artery. In this case, 5 ml of test substance was perfused into the abdominal artery at 2 ml min^{-1}, followed by saline.

In one preparation, stimulation at point A produced a biphasic response in the muscle fibers of the ipsilateral valve (Fig. 4C); in five others, stimulation at point B produced biphasic responses. In the illustrated record, low stimulation frequencies (<5 Hz) produced IJPs only. As stimulation frequency increased, the response became biphasic, with progressively shorter periods of hyperpolarization followed by depolarization. In most preparations, maximum depolarization occurred at a stimulating frequency of 40 Hz. In preparations exhibiting biphasic responses, the eventual loss of EJPs left only the IJPs in the ipsilateral and the contralateral valves.

Figure 5 illustrates that valve muscles receive dual inhibitory inputs that show facilitation when repetitively stimulated. These recordings were taken from the valve contralateral to the site of stimulation. A single 8-V stimulus resulted in a 1.5-mV hyperpolarization, while a 9-V stimulus recruited the second inhibitory axon and resulted in a 3-mV hyperpolarization. When stimulated by 8-V twin pulses (time delay between pulses = 170 ms), the first and second IJPs were 2 and 6 mV, respectively. Twin pulse stimulation at 9 V produced hyperpolarizations of 4 and 9.5 mV after the first and second pulses, respectively.

The bath application of a few drops of saline had no effect on membrane potential (Fig. 6A), but the same amount of ACh (10^{-5} *M*) caused a rapid depolarization of muscle fibers, with a gradual repolarization back to the resting membrane potential (Fig. 6B). GABA (10^{-5} *M*) elicited a rapid hyperpolarization of fiber membrane, with a slow recovery back to rest (Fig. 6C). The results shown are consistent with those taken from three animals. The IJPs were blocked by picrotoxin (Fig. 7). Fifty milliliters of 10^{-5} *M* picrotoxin was applied to the bath at a flow rate of 2.5 ml min^{-1} for 20 min, after which superfusion of the bath with saline resumed. Twenty minutes after the first application of picrotoxin, IJP magnitude was greatly reduced, and after 30 min it was virtually eliminated. Twenty minutes after resuming perfusion with fresh saline, IJP magnitude had partially recovered to pretreatment levels.

Spontaneous rhythmic oscillations in membrane potential of up to 28 mV and oscillation periods of 5–9 s were often observed (Fig. 8). These oscillations could persist for several hours and were usually accompanied by visible rhythmic contraction and relaxation of the valve flaps. The activity of spontaneously contracting LAAVs was modified by both ACh and GABA. ACh depolarized the valve muscle fibers, blocking the spontaneous oscillations in membrane potential; rhythmic oscillations gradually returned as ACh was washed out. Application of GABA to spontaneously oscillating muscle fibers produced a biphasic response: an initial period of membrane hyperpo-

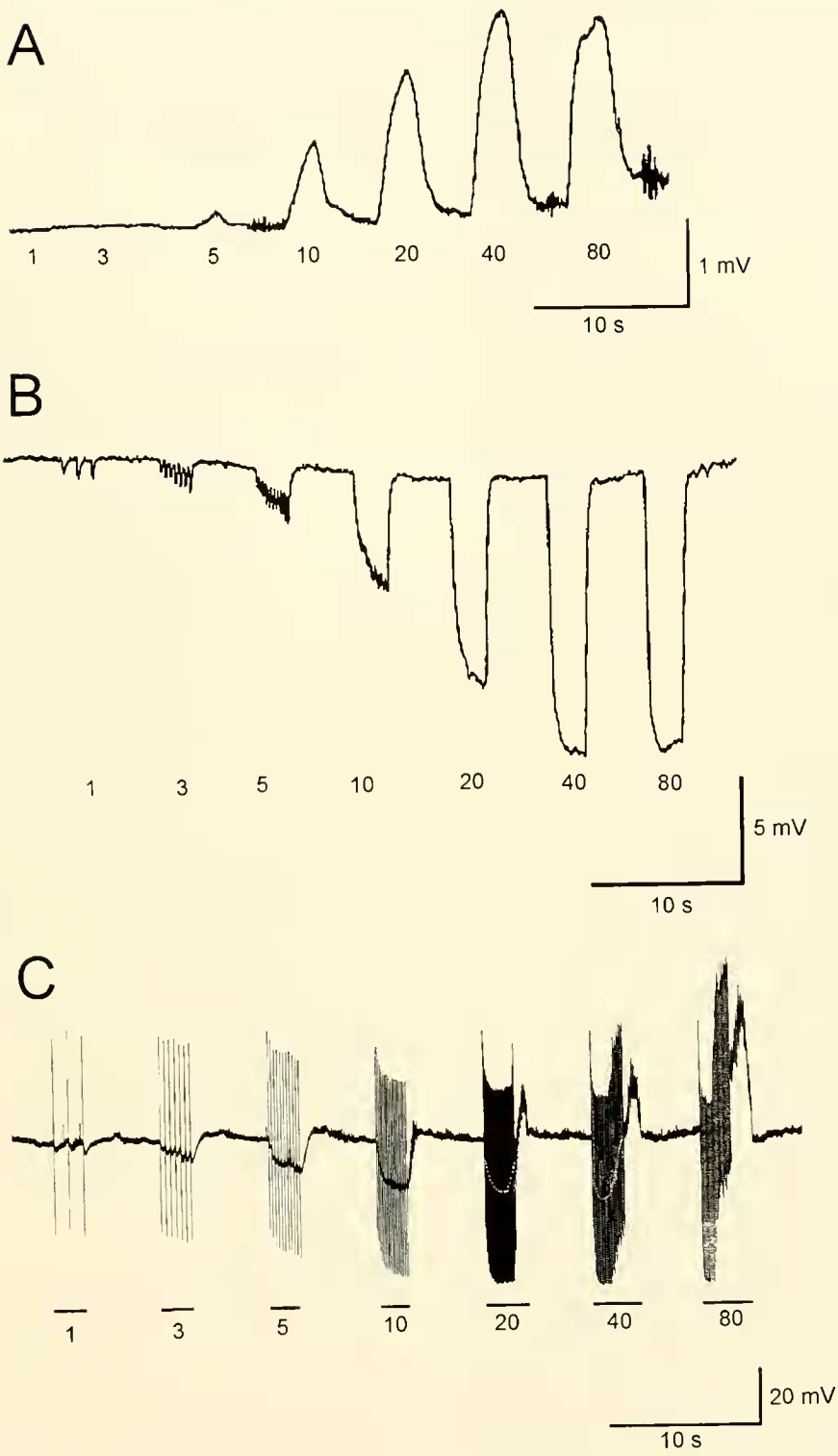

Figure 4. (A) Intracellular recordings from a valve muscle fiber during stimulation of the valve nerve at site A (Fig. 1C), and (B) at site B (Fig. 1C) at different frequencies. (C) Record of biphasic membrane potential responses to stimulation of valve nerve at site A (Fig. 1C). Only IJPs were produced below 5 Hz stimulation, summed IJPs followed by a small depolarization occurred at 5 and 10 Hz, and summed IJPs followed by repolarization during the period of stimulation and large EJPs occurred during stimulation at 20 Hz and higher. The vertical lines on each trace are stimulus artifacts. The inhibitory waveform during the 20 and 40 Hz stimulation was lost in these computer-scanned records; these two records are retouched to reveal the negative waveforms.

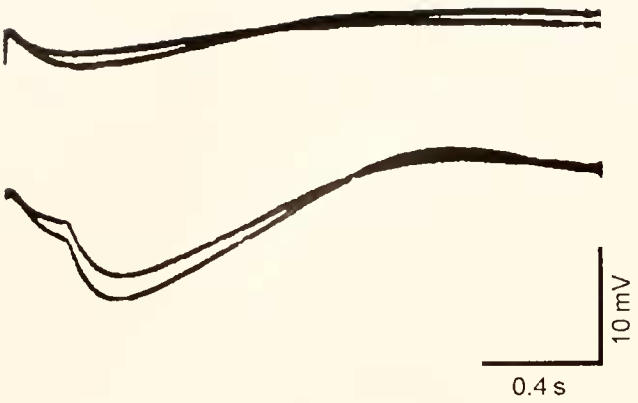

Figure 5. The effect of a single pulse (top trace) and twin pulse (lower trace, delay = 170 ms) stimulation of the lateral abdominal arterial valve nerve on muscle fiber membrane potential. Stimulation at two different voltages (8 and 9 V) in each pair of traces.

larization and decreased oscillation frequency, followed by a period of increased frequency of spontaneous depolarizations.

Discussion

This study focuses on the control of hemolymph distribution into and from the DAA, but to put these results into perspective relative to the overall control of hemolymph distribution, it is useful to review the general distribution of hemolymph among the seven arteries that leave the heart. Several crustacean species are known to have a muscular cardioarterial valve at the origin of each artery at the ventricle. These valves are in a position to control the distribution of cardiac output to large regions of the body (Kuramoto and Ebara, 1984; Kihara *et al.*, 1985; Fujiwara-Tsukamoto *et al.*, 1992; Kuramoto *et al.*, 1995). This holds true for *Homarus americanus*, except for the sternal and dorsal abdominal arteries that arise from the bulbus arteriosis at the back of the heart. The bulbus and ventricle are separated by a nonmuscular flap valve that serves to rectify hemolymph flow leaving the ventricle posteriorly (Kuramoto *et al.*, 1992). A different mechanism is responsible for partitioning of flow between these two posterior arteries. The outflow from the bulbus into the sternal artery is regulated by the muscular sternal cardioarterial valve (Kuramoto *et al.*, 1992, 1995), but there is no valve at the origin of the DAA. Instead the segmental LAAVs provide the only sites to regulate flow into and through the DAA. The DAA supplies hemolymph to the abdominal muscles and swimmerets through the segmental pairs of LAAs, and to the gut and gonads through a perfusion of small-diameter vessels that exit the ventrolateral wall of the DAA. Contraction of the LAAVs by neural (present results) or hormonal (Wilkens, 1997) inputs will decrease the flow into the DAA and redirect flow into other vascular beds, including those supplied by the small vessels to the gut and those supplied by the sternal artery. Conversely, a generalized neural-induced relaxation of the LAAVs will reduce the resistance of the DAA and favor an increased hemolymph flow into the LAAs that supply the abdominal muscles and swimmerets. Thus, LAAVs play important roles in controlling the distribution of hemolymph from the DAA.

The nerves supplying the LAAVs were first identified in *Potamobius astacus* by Alexandrowicz (1932a), who referred to them as the nervi segmentales aortae abdominalis. It is shown here that valve muscles of *H. americanus* receive both inhibitory and excitatory innervation. Stimulation of the inhibitory nerves causes hyperpolarization of valve muscle fibers and relaxation of the valves. This results in increased outflow through the LAAs. Valve fibers receive at least two inhibitory neurons. Excitatory nerves cause fiber depolarization, contraction of the valves, and reduction in LAA outflow. The sternal arterial valve in *H. americanus* appears to receive only inhibitory nervous input (Kuramoto *et al.*, 1992, 1995), a conclusion supported by the present results. One of the inhibitory neurons to this valve arises from an anterior projection from the LAA nerve from the first abdominal segment.

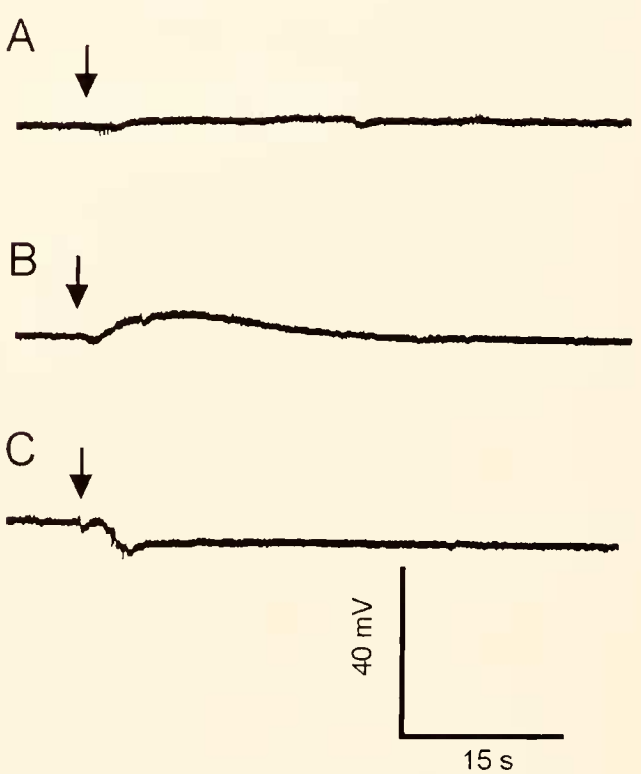

Figure 6. The effect on fiber membrane potential of adding 0.5 ml of (A) saline, (B) ACh (10^{-5} *M*), and (C) GABA (10^{-5} *M*) solution from a pasteur pipette directly over a lateral abdominal arterial valve muscle. Arrow indicates the time of application.

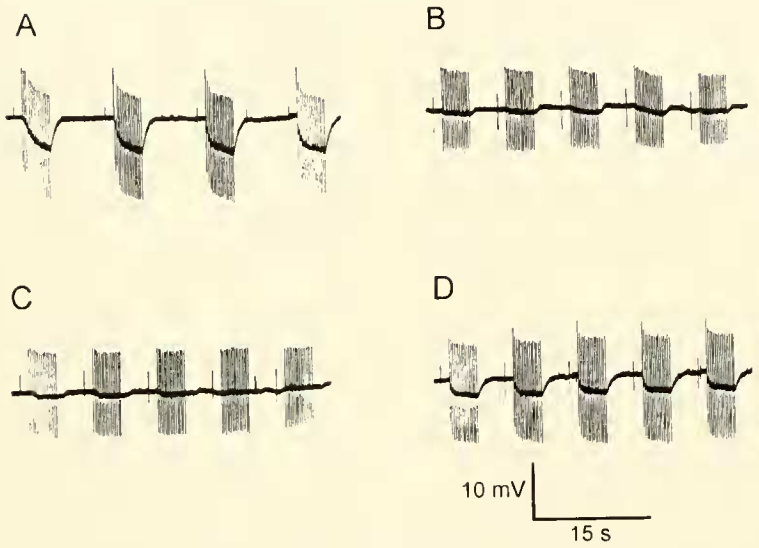

Figure 7. The effect of picrotoxin (10^{-5} *M*) on IJP amplitude during train stimulation (3.5-s train, 10 Hz) of the lateral abdominal arterial valve nerve. (A) Control responses; picrotoxin was added to the bath immediately after this recording. (B) Responses after 20-min exposure to picrotoxin. The bath was flushed with saline (2.5 ml min^{-1}) beginning at 20 min. (C) Recording after 10 min of saline wash. (D) IJP amplitude had partially recovered after 20 min of washing. The vertical lines on each trace are stimulus artifacts.

The nature of the innervation of the other cardioarterial valves in not known for lobsters. In the isopod *B. doederleini,* Kihara *et al.* (1985) found that some cardioarterial valves are dually and antagonistically innervated, whereas others receive only excitatory or inhibitory innervation.

It seems reasonable to assume that the complexity of the innervation of a valve is related to the degree of control required of that valve, and of the overall architecture of the arterial system of which the valve is a part. For example, the sternal artery in *H. americanus* supplies a number of critical structures, including the mouthparts, scaphognathites, walking legs, gut, and ventral nerve cord. It is unlikely that completely stopping hemolymph flow into this artery would ever be desirable, which may help explain the absence of excitatory nervous control of this valve. Flow into the sternal artery would, however, be indirectly reduced if the cardioarterial valves of other arteries and LAAVs were to dilate, lowering the relative flow resistances of other arterial pathways out of the heart. On the other hand, the DAA supplies two different vascular beds, and the complex control that includes both excitatory and inhibitory innervation would allow redistribution between them. We presume that the neural control of the LAAVs in *H. americanus* is related to swimmeret activity, as in *B. doederleini* (Fujiwara-Tsukamoto *et al.,* 1992), with increased inhibitory input during swimmeret and abdominal muscle activity and reduced inhibitory input during periods of low physical activity. Restriction of the flow out the LAAs by nervous and hormonal excitation of the valve muscles would favor flow into the smaller diameter and higher resistance gut vessels, presumably during digestive episodes.

The distribution of the LAAV nerves, with the projection of at least inhibitory axons to segments anterior to a particular nerve, may contribute to anterior waves of valve opening during anteriorly directed metachronal waves of swimmeret beating. However, the spontaneous membrane potential oscillations recorded *in vitro* seem unrelated to swimmeret activity because the frequency of the oscillations is lower than typical swimmeret beat frequencies (0.5–2 Hz; Cattaert and Clarac, 1983; Mulloney *et al.,* 1987; Barthe *et al.,* 1993), and the oscillations persist when the swimmerets are not active (see below). Further investigation is required to test this suggestion.

Biphasic changes in membrane potential were sometimes found during nerve stimulation, with a period of hyperpolarization preceding depolarization. The simplest interpretation of this observation is that both inhibitory and excitatory axons were stimulated and that the latency was shorter for the inhibitory inputs. This interpretation is similar to the time course of change in heart rate in crayfish (Wilkens and Walker, 1992; also seen in *H. americanus,* Wilkens, unpubl. obs.). At the heart, the response to cardioinhibitory nerve stimulation is quicker than that to cardioacceleratory nerve stimulation. Biphasic responses were not apparent in recordings of LAA flow and DAA pressure, where only the effects of valve contraction were apparent. A more detailed analysis, including the use of neurotransmitter blockers, will help understand the basis for these biphasic responses.

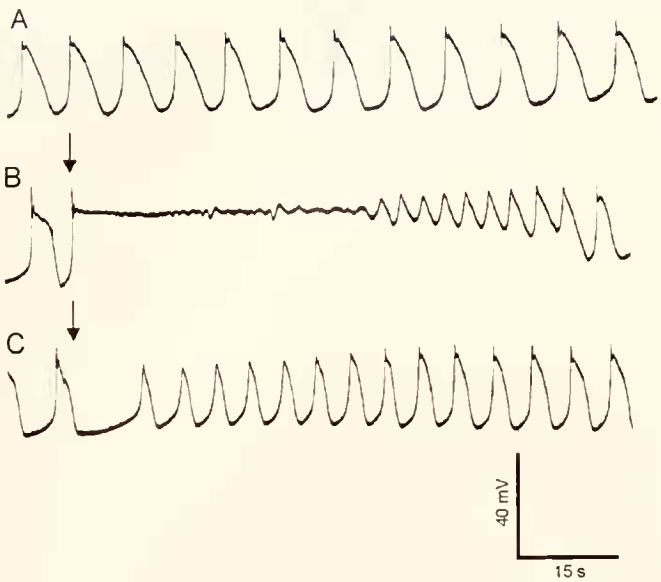

Figure 8. (A) Spontaneous membrane potential oscillations recorded from a lateral abdominal arterial valve muscle fiber when perfused with saline. (B) Application of 0.5 ml of ACh (10^{-3} *M*, arrow) depolarized the fiber and temporarily blocked the spontaneous oscillations. C) The application of 0.5 ml of GABA (10^{-3} *M*, arrow) at first prolonged the interoscillation interval and then increased the rate of oscillations. All records are taken from a single fiber.

It is most likely that GABA is the neurotransmitter released at the inhibitory LAAV neuromuscular junctions in *H. americanus*. This assumption is based on a number of observations. First, the magnitudes of hyperpolarizations of membrane potential resulting from nerve stimulation are similar to those produced by application of GABA. Second, during GABA-initiated hyperpolarization of LAAV muscle, electrical stimulation of the LAAV nerve had little effect, but as the membrane potential recovered from GABA, IJPs could again be elicited by nerve stimulation. Third, picrotoxin was effective in reversibly blocking nerve-induced and GABA-induced hyperpolarizations of the LAAVs. In another study, GABA hyperpolarized the sternal arterial valve and masked inhibitory postsynaptic potentials (IPSPs) arising from inhibitory nerve stimulation (Kuramoto *et al.*, 1992). In crustacean neuromuscular systems, picrotoxin depresses the increased membrane Cl^- conductance caused by GABA in a reversible noncompetitive manner (Takeuchi and Takeuchi, 1969). In view of the proposed homology between the sternal artery and LAAs (Wilkens *et al.*, 1997b), it is noteworthy that the nerve-induced hyperpolarization of the sternal cardioarterial valve is mimicked by dopamine and is not blocked by picrotoxin (Kuramoto *et al.*, 1992).

ACh, but not glutamic acid, caused depolarization of the valve muscle fibers, resulting in decreased flow through the LAA due to contraction of the LAAV. The magnitudes of these effects were similar to those caused by selective stimulation of the LAAV nerve, suggesting cholinergic innervation. This suggestion should be tested with further work using ACh blockers. The depolarization of valve fibers by ACh must account for the increase in the resistance to flow through the DAA (Wilkens, 1997). In *B. doederleini*, the inhibitory innervation of cardioarterial valves is cholinergic (Okada *et al.*, 1997). It appears that there is variability among the Crustacea in the physiological effects of ACh.

Spontaneous nonspiking oscillations in LAAV muscle membrane potential, with concurrent contraction and relaxation of the valves, were often observed in the present study. Spontaneous slow depolarizing and spiking potentials have been reported to occur in the sternal cardioarterial valve in *H. americanus* (Kuramoto *et al.*, 1992, 1995), and are produced in the cardioarterial valves of other arteries in both *H. americanus* and *Panulirus japonicus* (Wilkens and Kuramoto, unpubl. obs.). The records of arterial flow taken from intact *H. americanus* and *Cancer magister* often reveal cyclic fluctuations of flow (McMahon, 1992; McGaw *et al.*, 1994). Assuming that these rhythmic fluctuations are a result of rhythms of LAAV tension, we can ask about their physiological role. In the abdomen, the slow rhythmic opening and closing of the LAAVs may serve to maintain a low basal hemolymph flow to the abdomen and swimmerets during periods of locomotory inactivity and of increased digestive activity when it is predicted that the LAAVs would be closed.

Control of hemolymph distribution within a particular region would require peripheral mechanisms of flow control. Apart from the LAAVs of macrurans, very little is known about such peripheral sites of control of hemolymph flow in decapods. Taylor and Taylor (1986) identified structures in the gills of *Carcinus maenas* (L.) which they suggested might be efferent valves. In an abstract, Taylor *et al.* (1995) indicate that some cardioactive hormones can modulate vascular resistance to perfusion in crab gills *in vitro*. The resistances of perfused arterial trees in *H. americanus* (Wilkens, 1997) and *Procambarus clarkii* (Lovell and Wilkens, unpubl. data) are altered by several hormones.

Of the seven arteries leaving the heart in *H. americanus*, only the DAA has a layer of striated muscle in the artery wall (Wilkens *et al.*, 1997a). A muscular layer has also been identified in the dorsal abdominal arterial wall in the ridgeback prawn *Scyonia ingentis* (Martin *et al.*, 1989) and the spiny lobster *Panulirus interruptus* (Burnett, 1984). Indeed, this may be a common feature of all the macrurans. These muscle fibers are arranged circumferentially; their contraction will reduce the diameter of the vessel, and spontaneous rhythmic contractions of the DAA have been observed *in vitro* (Wilkens *et al.*, 1997b; Davidson and Lovell, unpubl. data).

Burnett (1984) postulated that the muscular DAA is a vestigial remnant of the tubular heart found in more primitive malacostracans, such as stomatopods. Wilkens *et al.* (1997b) supported this hypothesis and suggested in addition that the LAAVs may have been ancestral cardioarterial valves. In agreement with this suggestion, the anatomical pattern of innervation of the LAAVs in *H. americanus* is similar to that of the cardioarterial valves in the stomatopod *Squilla mantis* (Alexandrowicz, 1932b). The stomatopod cardiovascular system is thought to resemble a more primitive architecture (McLaughlin, 1980). In *S. mantis*, a branch of each of the segmental nerves supplying a cardioarterial valve passes across the heart to innervate the contralateral valve in the same segment, much in the same way that LAAV nerves innervate both valves within a segment in *H. americanus*. Wilkens *et al.* (1997b) further suggested that the sternal artery is homologous to the abdominal LAAs. This view is supported by observation that the anterior projection of the LAAV nerve in the first abdominal segment synapses on the sternal cardioarterial valve in the same manner that LAAVs in adjacent abdominal segments are connected. Interestingly, neural-induced relaxation of LAAVs in the first abdominal segment would presumably be accompanied by relaxation of the sternal cardioarterial valve due to their common innervation. Thus, in the absence of other control mechanisms such as hormones, changes in the resistances of the DAA and SA pathways may occur in parallel, rather than being regulated independently. There is no evidence that the sternal cardioarterial valve receives excitatory input.

The contraction and relaxation of the locomotory muscles in decapods may augment venous blood flow (Blatchford, 1971; Wood and Randall, 1981; McMahon and Burnett, 1990). Flow of dye injected into the ventral blood sinuses of the abdomen of *H. americanus* was shown to increase about 10-fold during abdominal flexion (Burger and Smythe, 1953). Extracardiac pumping would be possible only if valves are present to prevent the retrograde flow of blood. One function of the LAAVs may thus be to prevent blood backflow during the vigorous abdominal movements of swimming and escape tail flips. Passive flexion of the abdomen in semi-intact preparations of *H. americanus* causes a small rise in DAA resistance (Wilkens *et al.*, 1996), and tail flips cause pulses of increased pressure in the DAA in *P. clarkii* (Lovell and Wilkens, unpubl. obs.).

Taken together, the observations discussed here show that the LAAVs may be important in passively rectifying hemolymph flow and actively regulating the distribution of flow between the different vascular beds of the abdomen. It is yet to be investigated whether the nervous inputs to these valves are active as predicted during normal behaviors, and whether a similar valvular mechanism is involved in the control of peripheral resistance in the other arterial systems.

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